(19) Europäisch s Patentamt
European Patent Office
Office européen des brevets

(11) Publication number : **0 438 750 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication of patent specification : **27.10.93 Bulletin 93/43**

(51) Int. Cl.^{5} : **A61K 37/18**, A01N 63/02

(21) Application number : **90125035.7**

(22) Date of filing : **20.12.90**

(54) Lactoferrin hydrolyzate for use as an antibacterial agent.

Divisional application 92118531.0 filed on 20/12/90.

(30) Priority : **23.01.90 JP 13315/90**
26.06.90 JP 169636/90

(43) Date of publication of application :
31.07.91 Bulletin 91/31

(45) Publication of the grant of the patent :
27.10.93 Bulletin 93/43

(84) Designated Contracting States :
DE FR GB IT

(56) References cited :
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EP 0 438 750 B1

Description

The present invention relates to lactoferrin hydrolyzate for us as an antibacterial agent. In other words, the present invention relates to an antibacterial agent consisting of or containing lactoferrin hydrolyzate as the effective component.

Lactoferrin is known as an iron-binding protein occurring in lacrima, saliva, peripheral blood, milk and the like. It has been known that lactoferrin has antibacterial activity against coliform bacillus (Escherichia coli), Staphylococcus and other enterobacteria (or enteric bacteria) in a concentration within the range of 0.5 - 30 mg/mℓ (Nonnecke, B. J. and Smith, K. L.; Journal of Dairy Science; Vol.67, pp.606; 1984).

It has been considered in general that antibacterial activity of lactoferrin is derived from the situation wherein environmental iron becomes unutilizable to those microorganisms which require iron strongly due to the chelation of lactoferrin with environmental iron. The antibacterial activity of lactoferrin is not necessarily strong enough, thus a considerable quantity of lactoferrin is required to utilize its antibacterial activity, especially when lactoferrin is added to, impregnated into, sticked to, or coated onto other materials. Thus there was a limitation of its usefulness as an antibacterial agent.

It has been attempted to increase antibacterial activity of lactoferrin. For example, it is proposed to use lactoferrin together with lysozyme (Japanese Unexamined Patent Application Gazette No. 62(1987)-249931) . It has been also reported that copresence of lactoferrin and secretory IgA may multiplicatively augment antibacterial activity of the former (S. Stephens, J.M. Dolby, J. Montreuil and G. Spik; Immunology; Vol. 41; Page 597; 1980).

As far as the best knowledge of the inventors, however, there was no report indicating that chemical treatment of lactoferrin may improve its antibacterial activity.

It has been also known that lactoferrin is unstable to heating, and that the antibacterial activity of lactoferrin can be almost deactivated by heating it at 62.5 °C for 30 minutes, and complete deactivation is resulted by heating it at 70 °C for 15 minutes (Ford, J. E. et al; Journal of Pediatrics, Vol. 90, page 29; 1977).

Therefore, sufficient thermal treatment cannot be applied to those materials which contain lactoferrin as an antibacterial agent.

Also it has been known that lactoferrin is not stable to pH variation.

The inventors of the present invention have exerted their efforts to increase antibacterial activity of lactoferrin and to improve stability to heating, and found that hydrolyzate of lactoferrin substances such as native lactoferrin, apolactoferrin, metal saturated lactoferrin, and a mixture thereof show extremely stronger antibacterial activity and excellent stability to heating than unhydrolyzed lactoferrin. The present invention is based on this discovery. The words "native lactoferrin" used herein means that lactoferrin was just isolated from milk and the like, and that no chemical treatment such as iron removing and chelation with metals is made thereon.

In accordance with the present invention there is provided the use of lactoferrin hydrolyzate for the manufacture of an antibacterial agent characterized in that the lactoferrin hydrolyzate is obtainable by hydrolysis of lactoferrin. The preferable range in decomposition rate of lactoferrin hydrolyzate for use as an antibacterial agent is 6-20%, especially 7-15%, as measured by formol titration method (percentage of formol nitrogen to total nitrogen).

It should be noted that an additional effect of tyrosinase inhibition can be expected when 0.05% or more of lactoferrin hydrolyzate having decomposition rate of 6-20% is included in the antibacterial agent. This provision is the subject-matter of a divisional application.

Lactoferrin hydrolyzate can be prepared by the conventional method (for example, hydrolysis of lactoferrin by organic or inorganic acids or by enzymes).

Any lactoferrin substance can be used as the starting material for preparation of lactoferrin hydrolyzate, for example, lactoferrin obtainable in the market, native lactoferrin just isolated by the conventional method (for example, ion-exchange chromatography) from mammalian milk, apolactoferrin obtainable by removing iron from native lactoferrin with hydrochloric acid, citric acid and the like, metal saturated lactoferrin obtainable by chelating apolactoferrin with iron, copper, zinc, manganese and the like, or a mixture thereof (hereinafter these lactoferrin substances are abbreviated as LF).

Any mammalian milk (for example, human breast milk as well as cow's, sheep's, goat's, horse's milk and the like) at any lactation stage (for example, colostrum, transitional milk, matured milk, milk in later lactation) can be used as the source of LF. Furthermore, processed milk or byproducts in milk-processing such as skim milk, whey and the like can be used as the source of lactoferrin (hereinafter they are referred to as milk and the like).

Acid hydrolysis can be performed in accordance with th conventional methods, for example, LF is dissolved into water or purified water in a concentration within the range of 0.1 - 20 % (by weight, the same will be applied otherwise indicated), preferably 5 - 15 %, followed by pH adjustment of the resultant solution to 1

- 4, preferably to 2 - 3, and hydrolysis reaction at a proper temperature depending upon the pH of the solution. For instance, when the pH is adjusted to 1 - 2, the solution is heated at 80 - 130 °C, preferably at 90 - 120 °C; when pH is adjusted to 2 - 4, the solution is heated at 100 - 130 °C, preferably at 100 - 120 °C; respectively for 1 - 120 minutes, preferably 5 - 60 minutes until decomposition rate (by formol titration) of LF hydrolyzate is reached to 6 - 20 %, preferably to 7 - 15 %.

Enzymatic hydrolysis can be performed in accordance with the conventional method, for example, LF is dissolved into water or purified water in a concentration between 0.5 - 20 %, preferably 5 - 15 %, followed by pH adjustment of the resultant solution into the optimum pH range, and enzymatic hydrolysis under proper conditions, for example, a temperature between 15 - 55 °C, preferably between 30 - 50 °C for 30 - 600 minutes, preferably for 60 - 300 minutes. The reacted mixture is neutralized, followed by deactivation of the enzyme in accordance with the conventional method.

In the preparation of an antibacterial agent, any acidic enzymes such as Molsin F (trademark; by Seishin Seiyaku; optimum pH.: 2.5 - 3.0), swine pepsin (by Wakoh Junyaku Kogyo; optimum pH: 2 - 3), Sumizyme AP (trademark; by Shin Nihon Kagaku; optimum pH: 3.0), Amano M (trademark; by Amano Seiyaku; optimum pH: 3.0) and the like can be used individually or in any combination thereof. Among them, good results were obtained by use of swine pepsin and Sumizyme AP.

The quantity of enzymes to be used may be 0.1 - 5.0 %, preferably 0.5 - 3.0 % to the substrate used.

Regardless of the method of hydrolyzation, the resultant solution containing LF hydrolyzate is cooled by the conventional method, if necessary, followed by neutralization, demineralization, and decolorization. The resultant solution can be used as a liquid product as it is or if required, the solution is further concentrated and/or dried to obtain a concentrated liquid product or a powdery product.

The conditions of hydrolysis referred in the above are not critical, but can be modified depending upon the temperature, the period of time, the pressure as well as the kind and quantity of the acid or enzyme used.

LF hydrolyzate in the present invention is a mixture of the decomposed substances having different molecular weights.

Thus obtained LF hydrolyzate is highly stable to heating, and is excellent in antibacterial activity compared to unhydrolyzed lactoferrin.

Now some tests will be described hereunder for exemplifying the utility of LF hydrolyzate as an antibacterial agent.

[TEST 1]

The purpose of this test is to show relationships between decomposition rate of LF hydrolyzate and antibacterial activity.

1) METHOD

1-1) PREPARATION OF SAMPLES

Native LF sold in the market (by Oleofina, Belgium) was dissolved into purified water in 5 % concentration. The resultant solution was divided into several lots which were adjusted into different pH, 1, 2, 3 and 4 by adding 1 M hydrochloric acid thereto. The resultant solutions having different pH were subjected to hydrolysis reaction under different conditions in a combination of a temperature between 60 - 130 °C and a time between 5 - 60 minutes to thereby prepare samples of LF hydrolyzate having different decomposition rates as shown in Table 1.

1-2) MEASUREMENT OF HYDROLYZATION RATE

Decomposition rate (%) of the resultant LF hydrolyzate was determined in such a manner that the quantity of formol nitrogen in the respective samples was measured by formol titration method, then the resultant values were applied to the following formula.

$$\text{decomposition rate (\%)} = 100 \times (A / B)$$

(wherein A denotes the quantity of formol nitrogen, B denotes the quantity of total nitrogen)

1-3) PREPARATION OF PRE-CULTURE AND CULTURE MEDIUM

1-3-1) PREPARATION OF PRE-CULTURE

From the stock culture of Escherichia coli, a loop of bacterial cells was taken out with a platinum loop and smeared onto a standard plate agar medium (by Nissui Seiyaku) , followed by incubation at 35 °C for 16 hours under aerobic condition. The coloniss grown on the surface of the culture were collected with a platinum loop and suspended into sterilized saline solution to prepare pre-culture having optical density of 1.0 (at 660 nm) measured by a spectrophotometer (by Hitachi Seisakusho).

1-3-2) PREPARATION OF BASIC CULTURE MEDIUM

Basic culture medium (liquid culture medium) was prepared with dissolving bactocasitone (by Difco) into purified water in 1 % concentration, adjusting the pH of the resultant solution to 7.0 by 1 M sodium hydroxide, then sterilized at 115 °C for 15 minutes.

1-3-3) PREPARATION OF TEST AND CONTROL CULTURE MEDIA

The solutions of LF hydrolyzates previously prepared in item 1-1) were respectively filtrated with membrane filters (by Advantech) to remove microorganisms which might be included therein. Different quantities of each of filtrated solutions of LF hydrolyzates were respectively added to a portion of the basic culture medium to give 6 lots of test culture media containing LF hydrolyzate in different concentrations for each LF hydrolyzate having different decomposition rates as shown in Table 1. Control culture media containing unhydrolyzed LF in different concentration were also prepared in the same manner as in the test culture media.

1-3-4) TEST FOR ANTIBACTERIAL ACTIVITY

To each of the test and control culture media, the pre-culture was inoculated in 1 % concentration, followed by incubation at 35 °C for 16 hours. The proliferation inhibition rate was determined by measuring optical density of the culture broth in the same manner as previously described and calculated in accordance with the following formula.

$$\text{proliferation inhibition rate (\%)} = 100 - (A / B \times 100)$$

(wherein A denotes the difference of optical densities of the test culture media before and after 16 hours incubation, B denotes the difference of optical densities of the control culture media before and after 16 hours incubation respectively)

2) RESULTS OF THE TEST

The results are shown in Table 1.

Table 1

decomposition rate (%)	concentration (ppm)					
	25	50	100	250	500	1000
control	0	0	0	16	42	65
6	8	26	40	94	100	100
7	17	41	98	100	100	100
8	21	60	100	100	100	100
9	28	69	100	100	100	100
10	23	74	100	100	100	100
11	18	59	100	100	100	100
12	11	45	100	100	100	100
13	4	40	94	100	100	100
14	2	38	86	100	100	100
15	0	16	73	97	100	100
16	0	9	47	84	96	100
18	0	2	32	68	89	100
20	0	0	20	56	82	93
25	0	0	2	9	25	60
30	0	0	0	0	1	5

Note: the values indicate proliferation inhibition rates (%).

As will be seen from the Table 1, the unhydrolyzed LF (control) showed antibacterial activity when LF was added more than 250 ppm, but complete inhibition could not achieved even when 1000 ppm of LF was added (weak antibacterial activity). In contrast to this, LF hydrolyzate having 10 % decomposition rate showed potent antibacterial activity at the concentration of 25 ppm, and proliferation of E. coli was completely inhibited at a concentration over 100 ppm. It will be understood that LF hydrolyzate of decomposition rate between 6 - 20 %, especially between 7 - 15 %, prepared by acid hydrolysis of LF have remarkably potent antibacterial activity in comparison with unhydrolyzed LF.

[TEST 2]

Ten samples of powdery LF hydrolyzates, nos. 1 - 10, were prepared in such a manner that each of 5 kinds of commercial enzymes was added to 5 % aqueous solution of commercial LF (by Oleofina), followed by adjustment of pH to the optimum pH of the respective enzymes, enzymatic hydrolyzation at 37 °C for different reaction times, adjustment of pH of the hydrolyzed solutions to 7, deactivation of the enzymes at 80 °C for 10 minutes, and lyophilization of the resulted solutions. The kinds of enzymes and their quantities added to the substrate (LF), reaction times of hydrolyzation and decomposition rates of the respective samples are shown in Table 2. The decomposition rates were determined by the same method as in Test 1.

2) RESULT

The results of this test are shown in Table 2.

Table 2

sample No.	enzymes used	quantity of enzyme (%)	reaction time (minutes)	decomposition rate (%)
1	Molsin F	0.1	120	12.4
2	Molsin F	1.0	300	14.7
3	swine pepsin	0.3	30	6.3
4	swine pepsin	3.0	180	11.4
5	Sumizyme AP	1.0	180	10.3
6	Sumizyme AP	3.0	180	13.5
7	Amano M	0.1	60	5.9
8	Amano M	3.0	120	12.6
9	Trypsin	3.0	180	10.3
10	Trypsin	6.0	360	12.5

As will be seen from Table 2, decomposition rates of LF hydrolyzate by acidic protease such as Molsin F, swine pepsin, Sumizyme AP, and Amano M fallen between 5.9 - 14.7 %, and those by trypsin which is a neutral protease were 10.3 - 12.5 %.

[Test 3]

The purpose of this test is to exemplify antibacterial activity of LF hydrolyzate prepared by acid hydrolysis.

Antibacterial activity against E. coli of LF hydrolyzate having 12 % decomposition rate prepared in the same manner as in Example 2 (hydrolyzation by citric acid) and that of unhydrolyzed LF were defermined in the same manner as in Test 1.

The results are shown in Table 3.

Table 3

sample	quantity added (ppm)					
	25	50	100	250	500	1000
unhydrolyzed LF	0	0	5	16	74	85
LF hydrolyzate	12	41	100	100	100	100

Note: The values indicate proliferation inhibition rates (%).

From the results of the foregoing test, it is exemplified that LF hydrolyzate prepared by organic acid hydrolysis, and inorganic acid hydrolysis have antibacterial activity.

[TEST 4]

The purpose of this test is to exemplify effectivity of the method for treatment of a material with antibacterial agent.

A quantity of sliced vegetables sold in the market was divided into three parts, each of the parts was respectively dipped into 1 % aqueous solution of LF hydrolyzate prepared in the same manner as in Example 1 (hydrolyzed by inorganic acid, decomposition rate: 9 %), unhydrolyzed LF solution (control) and sterilized water

(control) for 30 seconds. Each of the treated samples was drained then preserved at 5 °C for observation. Viable bacterial count of the samples was periodically determined by the conventional method.

The results of this test are shown in Table 4.

Table 4

Samples	preservation period (hours)			
	0	12	24	36
sterilized water	5.1×10^3	4.9×10^4	5.0×10^5	9.2×10^5
unhydrolyzed LF	5.1×10^3	2.0×10^4	1.8×10^5	2.2×10^5
LF hydrolyzate	5.1×10^3	1.1×10^3	1.5×10^3	1.6×10^3

Note: The values indicate viable bacterial counts per 1 g of sliced vegetables.

As will be seen from the Table 4, it is exemplified that LF hydrolyzate has remarkably stronger antibacterial activity than unhydrolyzed LF when they are used for treatment of materials.

[Test 5]

The purpose of this test is to exemplify the effectiveness of inclusion of LF hydrolyzate in a food as the effective component for antibacterial activity.

A quantity of raw milk was sterilized at 65 °C for 30 minutes and dispensed into test tubes by 10 mℓ. To each of the test tubes, LF hydrolyzate having 9 % decomposition rate (prepared in Example 1) or unhydrolyzed LF were added and homogeneously mixed in 0.1 % concentration, and sealed to prepare samples No. 1 and No. 2. A sample which contains raw milk only was prepared as a control sample. These samples were preserved at 25 °C to determine preservable days during which coagulation of raw milk was not observed.

The results were that coagulation was observed in sample No. 1 on the 9th day, in sample 2 on the 4th day, and in control sample on the 2nd day. This means that LF hydrolyzate has excellent antibacterial activity. Meanwhile, organoleptic test was carried out with respect to the control sample and sample No. 1 immediately after preparation of the samples, and confirmed that there was no difference in taste and appearance therebetween.

[TEST 6]

The purpose of this test is to exemplify antibacterial activity of LF hydrolyzate prepared by enzymatic hydrolysis.

1) METHOD

Aqueous solutions of 10 kinds of LF hydrolyzate (prepared using different enzymes) and an aqueous solution of unhydrolyzed LF prepared in the same manner as in test 2 were filtrated with membrane filters (by Advantec) to remove bacterial cells which might be contaminated therein. Each of the solutions were added to basic culture media (the same with that in Test 1) in different concentrations (50, 100, 250, 500, and 1000 ppm) to prepare test cultures and control cultures.

Antibacterial activity was tested for these cultures in the same manner as in test 1.

2) RESULTS

The results are shown in Table 5.

Table 5

sample No.	proliferation inhibition rate (%)				
	quantity of LF hydrolyzate added (ppm)				
	50	100	250	500	1000
1	20	78	100	100	100
2	2	54	96	100	100
3	0	5	24	78	100
4	41	97	100	100	100
5	35	93	100	100	100
6	16	62	100	100	100
7	0	3	15	38	61
8	26	65	84	100	100
9	0	0	0	0	0
10	0	0	0	0	0
control	0	0	7	25	46

As will be seen from Table 5, unhydrolyzed LF (control) showed weak antibacterial activity at addition of 250 ppm, and complete inhibition against proliferation of E. coli could not be achieved even by addition of 1000 ppm. LF hydrolyzate having decomposition rate over 10 % resulted from hydrolysis by Molsin F, swine pepsin, Sumizyme AP and Amano M all of which are acidic protease showed potent antibacterial activity at presence of only 100 ppm, and presence over 250 ppm may inhibit proliferation of coliform bacillus perfectly. On the other hand, LF hydrolyzate resulted from hydrolysis by trypsin which is a neutral protease did not show any antibacterial activity even at presence of 1000 ppm.

It was exemplified that LF hydrolyzate resulted from hydrolysis by acidic protease having decomposition rate more than 10 % has stronger antibacterial activity against coliform bacillus than unhydrolyzed LF.

[TEST 7]

The purpose of this test is to confirm antibacterial activity of a composition containing hydrolyzate of metal saturated LF.

Antibacterial activity of hydrolyzate of iron saturated LF was tested in the same manner as in test 5, except that hydrolyzate of iron saturated LF prepared in the same manner as in Example 5 and cow's milk sold in the market were used.

Three kinds of samples, one with added hydrolyzate of Fe-LF, another one with added unhydrolyzed LF and one with no addition (control), were respectively distributed into 3 test tubes (in total 9) and preserved at 25 °C for observation of any change in appearance.

The results are shown in Table 6.

Table 6

sample		preservation (day)				
		0	3	7	10	14
hydrolyzate of Fe-LF	1	no change	no change	no change	no change	no change
	2	no change	no change	no change	no change	no change
	3	no change	no change	no change	no change	no change
unhydrolyzed Fe-LF	1	no change	no change	no change	no change	coagulated
	2	no change	no change	no change	no change	coagulated
	3	no change	no change	no change	no change	coagulated
control (added nothing)	1	no change	no change	no change	coagulated	coagulated
	2	no change	no change	no change	no change	coagulated
	3	no change	no change	no change	coagulated	coagulated

All of the samples (milk) added hydrolyzate of Fe-LF did not show any change in appearance on the 14th day after initiation of the test. All of the samples added unhydrolyzed LF did not show any change in appearance until 10th day after initiation of the test, but all of the samples showed coagulation on the 14th day. In control samples, 2 samples showed coagulation on the 10th day, and all of the samples coagulated on the 14th day. It was exemplified that hydrolyzate of Fe-LF have excellent antibacterial activity in comparison with unhydrolyzed LF.

[TEST 8]

The purpose of this test is to confirm antibacterial activity of LF hydrolyzate under the presence of iron.

Antibacterial activity was tested with respect to some samples used in Test 6, i. e. sample Nos. 1, 2, 5, 6 and the sample containing unhydrolyzed LF (control) in the same manner as in Test 6, except that 0.01 mM of ferrous sulfate ($FeSO_4$) was further added to the respective culture media.

The results are shown in Table 7.

Table 7

sample No.	proliferation inhibition rate (%)				
	quantity of LF hydrolyzate (ppm)				
	50	100	250	500	1000
1	13	59	100	100	100
2	0	26	87	100	100
5	27	65	86	100	100
6	14	40	93	100	100
control	0	0	0	0	0

Antibacterial activity of unhydrolyzed LF was deactivated under the presence of 0.01 mM of ferrous sulfate, however, all of the LF hydrolyzate resulted from hydrolyzation by acidic protease maintained its antibacterial activity under the presence of 0.01 mM of ferrous sulfate.

Now, some examples will be described for better understanding of the present invention.

EXAMPLE 1

To 950 g of purified water, 50 g of native LF sold in the market (by Ol ofina, Belgium) was dissolved, then the pH of the resulted solution was adjusted to 2 with 1 M of hydrochloric acid. The resultant solution was heated to 120 °C for 15 minutes for acid hydrolysis, then cooled, thereby about 1000 g of 5 % solution of LF hydrolyzate having antibacterial activity was obtained. The decomposition rate of the LF hydrolyzate was 9 % (determined by the same method as in Test 1).

EXAMPLE 2

To 850 g of purified water, 150 g of native LF sold in the market (by Oleofina, Belgium) was dissolved. The resultant solution was adjusted to pH 3 with 1 M of citric acid, then heated to 130 °C for 60 minutes for hydrolyzation. The resulted solution was cooled, adjusted to pH 7 with 1 M sodium hydroxide, filtrated, demineralized, then lyophilized (freezedried) thereby about 45 g of powdery LF hydrolyzate having antibacterial activity was yielded.

The decomposition rate of this LF hydrolyzate was 12 % (determined by the same method as in Test 1).

EXAMPLE 3

1) PREPARATION OF Fe SATURATED LF

To 180 g of purified water, 20 g of native LF sold in the market (by Oleofina, Belgium) was dissolved. To the resultant solution, 200 mg of $FeSO_4 \cdot 7H_2O$ was added. After maintaining at 25 °C for 12 hours, unreacted Fe was removed from the reacted solution by ultrafiltration module SEP-1013 (trademark, by Asahikasei), then the resultant filtrate was lyophilized to thereby obtain about 19 g of iron saturated LF.

2) HYDROLYSIS OF Fe SATURATED LF

To 285 g of purified water, 15 g of iron saturated LF was dissolved, the pH of the resulted solution was adjusted to 1.0 with 2 M hydrochloric acid, heated at 90 °C for 15 minutes for hydrolysis, then cooled, thereby about 300 g of about 5 % solution of LF hydrolyzate having antibacterial activity was yielded.

The decomposition rate of the resulted LF hydrolyzate was 7 % (determined by the same method as in Test 1).

EXAMPLE 4

1) PREPARATION OF COLUMN

In a column (id. 10 cm), 500mℓ of Sepabeads FP-CM13 (trademark, by Mitsubishi Kasei) having a carboxymethyl group as an ion-exchange group was filled, then 10 % aqueous solution of sodium chloride was passed through the resulted column. The column was washed with water to thereby obtain Na-type ion-exchanger.

2) PREPARATION OF NATIVE LF

To the resultant column, 60 ℓ of cheese whey (pH 6.5) originated from goat milk was introduced at 4 °C at the flow rate of 4 ℓ/hour. After the column was washed with water to remove the unadsorbed components of the cheese whey, the adsorbed component of the cheese whey was eluted with 10 % aqueous solution of sodium hydroxide at the flow rate of 5 ℓ/hour to thereby obtain about 5 ℓ of eluate. The resultant eluate was concentrated with ultra-filtration module SEP-1013 (trademark, by Asahikasei) then water was added thereto to remove sodium chloride to thereby obtain about 200 mℓ of LF solution containing 1 % goat LF.

3) ACID HYDROLYSIS

The resultant LF solution was adjusted to pH 2.0 with 1 M hydrochloric acid, heated to 120 °C for 20 minutes, then cooled, to thereby obtain about 200 g of 1 % solution of LF hydrolyzate having antibacterial activity. The decomposition rate of the LF hydrolyzate was 10 % (determined by the same method as in Test 1).

EXAMPLE 5

1) PREPARATION OF Fe SATURATED LF

To 9 kg of purified water, 1 kg of native LF (by Oleofina, Belgium) was dissolved. To the resultant solution, 10 g of $FeSO_4 \cdot 7H_2O$ was added, maintained at 25 °C for 12 hours. From the resultant solution, unreacted iron was removed by ultrafiltration module SEP-1013 (trademark, by Asahikasei).

2) ENZYMATIC HYDROLYSIS

After adjusted the resultant solution to pH 3.5 with 0.5 N hydrochloric acid, 10 g of Molsin F (trademark, by Seishin Seiyaku, 42,000 unit/g of protein) sold in the market was added to the solution and homogeneously mixed. The resultant mixture was maintained at 37 °C for 180 minutes, neutralized, heated to 85 °C for 10 minutes for deactivation of the enzyme, then cooled, thereby about 10 kg of LF hydrolyzate solution having antibacterial activity was yielded.

The decomposition rate of the resultant LF hydrolyzate was 13.5 % (determined by the same method as in Test 1).

EXAMPLE 6

To 9 kg of purified water, 1 kg of native LF (by Oleofina, Belgium) was dissolved. The resultant solution was adjusted to pH 2.5 with 2 M citric acid, 30 g of swine pepsin sold in the market (10,000 unit/g of protein: by Wakoh Junyaku Kogyo) was added, the solution was homogeneously mixed, maintained at 37 °C for 180 minutes for hydrolysis, heated to 85 °C for 10 minutes for deactivation of the enzyme, cooled, concentrated by the conventional method to thereby obtain about 10 kg of a solution of the LF hydrolyzate having antibacterial activity.

The decomposition rate of the resultant LF hydrolyzate was 11.3 % (determined by the same method as in Test 1).

EXAMPLE 7

1) PREPARATION Cu SATURATED LF

Copper saturated LF was prepared as follows. To 50 ℓ of goat skim milk, 5 ℓ of 0.1 M citric acid solution containing 0.003 M ferric chloride ($FeC\ell_3$) was added and homogeneously mixed. To the resultant solution, 5 ℓ of CM-Sephadex C-50 (H^+ type, by Pharmacia) was added and stirred for 1 hour. After removing unadsorbed components of the goat skim milk by washing the ion-exchange resin with water, the resin was suspended in 0.05 M tris-hydrochloride buffer solution (pH 8.0). The resultant suspension was filled in a column (20 x 50 cm), then washed with the same buffer solution. The adsorbed components of goat skim milk were eluted with 0.05 M tris-hydrochloric acid buffer solution containing sodium chloride in a gradient of 0 - 2 M to thereby collect about 800 mℓ of LF fraction. The resultant LF fraction was concentrated into 150 mℓ with ultrafiltration membrane PM-10 (trademark, by Amicon), then dialyzed against 0.05 M tris-acetate buffer solution (pH 8.2) containing 0.5 M sodium chloride. The resultant dialyzed solution was introduced into a column (10 x 30 cm) filled with copper chelating Sephalose 6B (trademark, by Pharmacia) which was previously equilibrated with the same buffer solution to adsorb LF. After washing the column with the same buffer solution, the adsorbate was eluted with acetate buffer solution (pH 4.0) containing 0.5 M sodium chloride. The resultant eluate was dialyzed against purified water, then lyophilized to thereby obtain about 2 g of powdery LF.

2) ENZYMATIC HYDROLYSIS

To 17 g of purified water, 2 g of the resulted powdery LF was added, the resultant solution was adjusted to pH 3.5 with 1 M lactic acid. To the resultant solution, 60 mg of Sumizyme AP sold in the market (trademark, by Shinnihon Kagakukougyo, 50,000 unit/g of protein) was homogeneously added, maintained at 50 °C for 180 minutes, neutralized, then resultant reaction mixture was heated to 85 °C for 10 minutes for deactivation of the enzyme, cooled, concentrated then lyophilized to thereby obtain about 2 g of powdery LF hydrolyzate having antibacterial activity.

The decomposition rate of the resulted LF hydrolyzate was 13.8 % (determined by the same method as in test 1).

In summary, it can be concluded that:
1) LF hydrolyzat for use as an antibacterial agent is safe for human and animals, since it is a natural antibacterial substance derived form hydrolysis of milk components and the like.
2) LF hydrolyzate for use as an antibacterial agent has extremely stronger antibacterial activity than unhydrolyzed LF.
3) LF hydrolyzate for use as an antibacterial agent is stable to heating, and can be provided in liquid and powdery forms, thus it has wider application.
4) The antibacterial agent comprising LF hydrolyzate can be prepared by mixing with one or more of excipients or other medicines, inclusive of other antibacterial agents.
5) The antibacterial agent consisting of or comprising LF hydrolyzate can be utilized as a component of various products such as cosmetics, foods, feeds and other products which are desirable to be prevented or inhibited from qualitative deterioration due to proliferation of microorganisms. It is specifically noted that inclusion of the antibacterial agent consisting of or comprising LF hydrolyzate in such products is effective not only for preservation of the products, but also effective for therapy of bacterial infection or prevention thereform when the products are given to human and other animals or applied to the body surface thereof.
6) The antibacterial agent consisting of or comprising LF hydrolyzates can be utilized for treatment of various materials, for example washing or dipping the materials in a solution of the agent so that the agent is sticked to or coated onto or impregnated into those materials, for maintenance of sanitary conditions and for prevention from deterioration in freshness thereof. It is specifically noted that the materials treated are also effective for therapy against bacterial infection, prevention therefrom, when they are taken into or applied onto the body surface of human or animals subject to that effective amount of LF hydrolyzate is accompanied therewith (or remained therein).

Claims

1. Use of lactoferrin hydrolyzate for the manufacture of an antibacterial agent.
2. The use according to claim 1 wherein the lactoferrin hydrolyzate is obtained by hydrolysis of lactoferrin.
3. The use according to claim 1 or 2 wherein the decomposition rate of said hydrolyzate is 6-20%.
4. The use according to any preceding claim wherein at least 25 ppm of said hydrolyzate is included in said antibacterial agent.

Patentansprüche

1. Verwendung von Lactoferrinhydrolysat zur Herstellung eines antibakteriellen Mittels, dadurch gekennzeichnet, daß Lactoferrinhydrolysat durch Hydrolyse von Lactoferrin erhalten wird.
2. Verwendung nach Anspruch 1, bei welcher das Lactoferrinhydrolysat durch Hydrolyse von Lactoferrin erhalten wird.
3. Verwendung nach Anspruch 1 oder 2, bei welcher die Abbaurate des Hydrolysats 6 bis 20 % beträgt.
4. Verwendung nach einem der vorstehenden Ansprüche, bei welcher mindestens 25 ppm des Hydrolysats in dem antibakteriellen Mittel enthalten sind.

Revendications

1. Utilisation d'un hydrolysat de lactoferrine pour la préparation d'un agent antibactérien, caractérisée en ce que l'hydrolysat de lactoferrine peut être obtenu par hydrolyse de la lactoferrine.
2. Utilisation selon la revendication 1, dans laquelle l'hydrolysat de lactoferrine est obtenu par hydrolyse de la lactoferrine.
3. Utilisation selon la revendication 1 ou 2, dans laquelle le taux de décomposition dudit hydrolysat est de

6 à 20 %.

4. Utilisation selon l'une quelconque des revendications précédentes, dans laquelle au moins 25 ppm dudit hydrolysat sont incorporées audit agent antibactérien.